SINGLE NEURON OBSERVATION IN A 3D NEURONAL TISSUE BLOOK

Midori Kato-Negishi[1, 2], Hiroaki Onoe[1, 2] and Shoji Takeuchi[1, 2]

[1]Institute of Industrial Science, the University of Tokyo, Japan, [2]Takuchi Biohybrid Innovation Project, Exploratory Research for Advanced Technology (ERATO), Japan Science and Technology (JST), Japan

ABSTRACT

This paper describes a method to observe a single neuron in a three-dimensional (3D) neuronal tissue formed by bottom-up tissue construction. We first formed GFP-actin-transfected neurospheroids for visualization of neurons, and then formed a millimeter-sized neuronal block using PDMS mold chambers. We found that GFP-actin-transfected neurons in the neuronal block have *in vivo*-like morphology and 3D extension of their axons/dendrites compared to 2D culture system, and that many spines were successfully formed on the dendrites. We are confident that our neuronal block enables morphological maturation of the neurons due to its *in vivo*-like 3D environment, and that our bottom-up construction to observe single neurons is a powerful approach for various fields including analysis of single neuronal behaviors *in vivo*, and pharmacological assay for drug screening.

KEYWORDS

3D tissue engineering, Neuron, Cortex, GFP-actin, Transfection

INTRODUCTION

Single cell observation in neuronal tissues is needed to understand the mechanism of neuronal maturation, synapse formation, synaptic plasticity, and neuronal network formation. GFP-actin transfection system in slice tissue culture has been used for this purpose. But slice tissue culture system is not easy and inconvenient, since the tissue is dissected from a fresh brain every time. Therefore, 3D neuronal tissue fabrication methods have been developed [1-3]. Although 3D hydrogel culture is a common method, it is difficult to form *in vivo*-like neuronal morphology and many synapses. Here, we describe a method to observe a single neuron in a millimeter-sized neuronal tissue formed by bottom-up tissue construction. We also evaluated the morphological maturation of the neurons in the neuronal block and compared to 2D culture system.

Figure 1 shows the concept of our study, which consists of the following 3 steps: (1) Formation of GFP-actin-transfected neurospheroids, (2) Formation of neuronal block, and (3) Neuronal maturation and single cell observation. We have already reported the establishment of the neuronal block using PDMS mold chamber [4]. In this paper, we investigated in detail about the fabrication of neuronal block for observation of single cells.

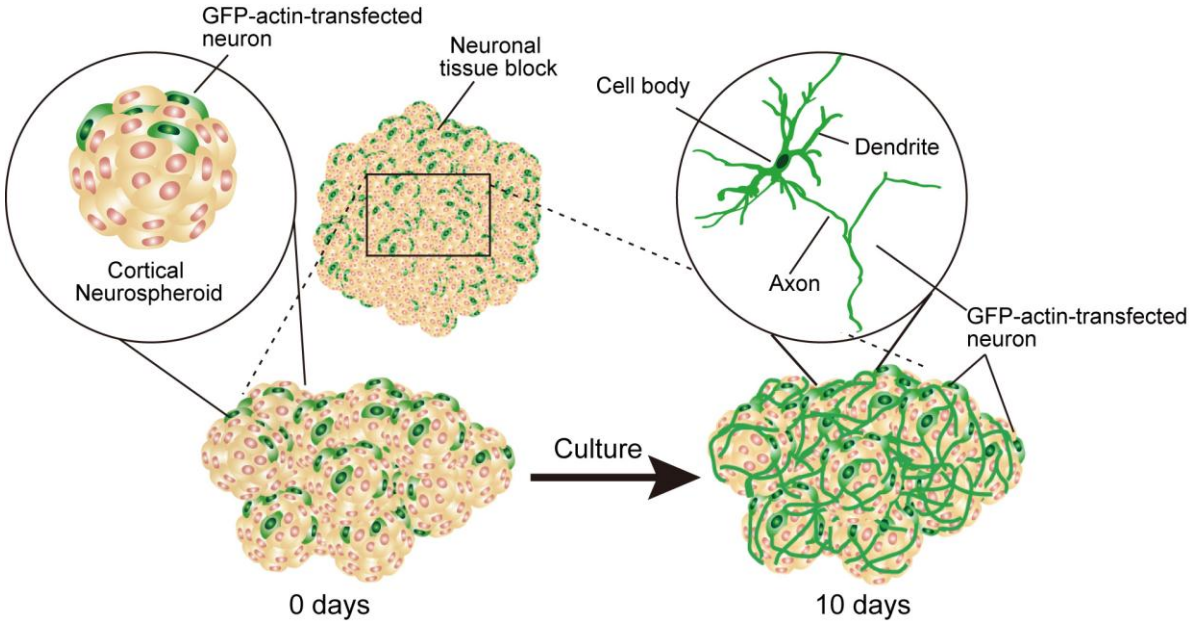

Figure 1. *Conceptual illustration of our study. We made a neuronal tissue block using "bottom up" neuronal tissue fabrication methods. In the neuronal block, we can observe the detailed morphological change of GFP-actin transfected neurons.*

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FORMATION OF NEURONAL BLOCKS

To fabricate a neuronal tissue block, we first prepared the uniform-sized neurospheroids on a PDMS microchamber array using the previously reported method [2]. PDMS microchamber array is generated using conventional photolithography and soft lithography techniques. Briefly, SU-8 negative photoresist were span onto a silicon wafer and soft baked. The resist was exposed to UV light through a patterned photomask using a mask aligner and developed using SU8-developer solution. This process creates an SU8 master that could be used to mold PDMS. The PDMS microchamber was formed by pouring PDMS (10:1, prepolymer: curing agent) onto the master and curing for 2 h at75 °C. Removal of the PDMS from the wafers yielded PDMS microchamber array for cell culture (Figure 2a).

The cerebral cortices were dissected from Wistar rats (embryonic day 16-19) and cultured using previously described methods [3-5]. Briefly, the cerebral cortices were dissociated with papain and triturated through a pipette. The cortical and hippocampal cell suspensions were seeded onto the PDMS microchamber array (diameter: 100 or 150 μm) and non-dropped cells were removed after 30 min by changing the medium. Cultured cells were maintained at 37°C under a humidified atmosphere of 5% CO_2 and 95% air. After 48-72 h of culture, we molded uniform-sized neurospheroids into a PDMS mold chamber and cultured (Figure 2b, c). Almost all neurospheroids connected to each other after 60 min of culture (Figure 3a). After 24 h of culture, millimeter-sized neuronal tissue blocks could be formed and completely released from the chamber (Figure 3a). We also estimated the shrinkage of a neuronal block in the following culture days (Figure 3b). Neuronal tissue blocks were shrunk to about 80 % by day 17.

SINGLE NEURON OBSERVATION

For the detailed observation of neuronal morphology in the 3D neuronal tissue block, we first transfected cortical neurons in the neurospheroids with pAcGFP1-actin vector using the Xfect™ transfection reagent (Figure 4). And then, millimeter-sized neuronal tissue blocks were formed by GFP-actin-transfected neurospheroids. After 10 days of culture, we observed GFP-actin-labeled single neuron in the neuronal tissue block using confocal laser microscope. GFP-actin-labeled neurons had *in vivo*-like morphology, and many spine-like structures were observed on their dendrites. We also compared dendrite and axonal extension between 2D neuronal culture condition and 3D neuronal tissue block (Figure 5). In the 3D neuronal tissue block, neurons extended their dendrites and axons at Z-depth of more than 120 μm.

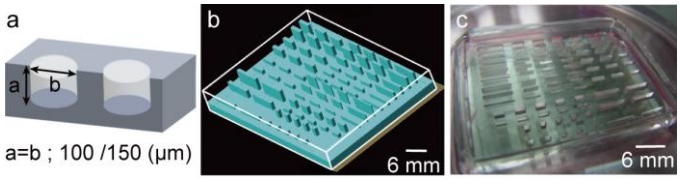

Figure 2. *Microchamber and mold. (a) Illustration of PDMS microchamber. The diameter and depth of the chamber had the same value. (b) 3D illustration of a mold to produce the neuronal blocks. (c) Photograph of a PDMS chamber to produce the neuronal blocks.*

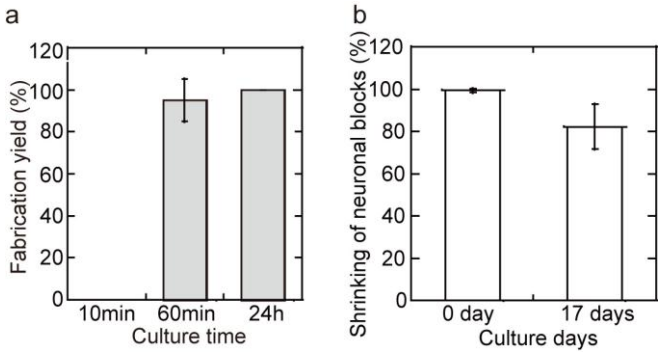

Figure 3. *Formation and shrinkage of neuronal tissue block. (a) Fabrication yield of neuronal blocks at 10 min, 60 min. and 24 hours. (d) Shrinking change of neuronal tissue blocks after 17 days of culture.*

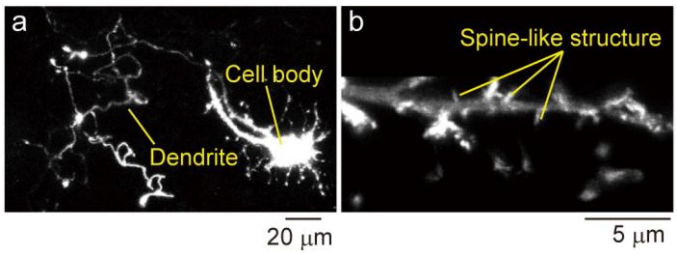

Figure 4. *GFP-actin-transfected neurons in a neuronal tissue block. (a) Confocal laser microscopic image of GFP-actin-transfected neurons in the neuronal tissue block. (b) Confocal laser microscopic image of a dendrite in the neuronal tissue block. Dendrite had many spine-like structures.*

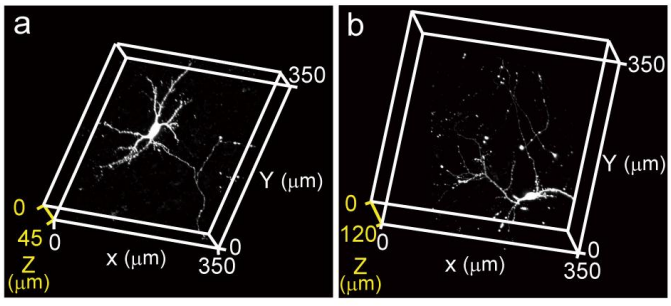

Figure 5 *GFP-actin transfected neurons in the 2D culture and neuronal blocks. (a), (b) Confocal laser microscopic images of GFP-actin transfected neurons.*

CONCLUSION

This study describes a strategy for single cell observation in a 3D neuronal tissue block. We developed an efficient technique to visualize neuronal morphology in millimeter-sized neuronal tissue blocks. We also demonstrated that many spine-like structures were observed on the dendrites in the neuronal tissue block, and dendrites and axons were extended in 3D spatial-space. Therefore, single neuron observation system would be an extremely effective tool for the analysis of single neuronal behaviors in 3D neuronal tissue.

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CONTACT

Midori Kato-Negishi, Institute of Industrial Science, the University of Tokyo, 4-6-1, Komaba Meguro-ku, Tokyo, JAPAN, Tel: +81-3-5452-6650; Fax: +81-3-5452-6649, E-mail: negishi@iis.u-tokyo.ac.jp